



## 2014–2016 seasonal rainfall effects on metals in tea (*Camelia sinensis* (L.) Kuntze)

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### H I G H L I G H T S

- Metal nutrient concentrations in tea varied in response to climate changes.
- Concentrations of Fe, Ca, Mg, Mn, Al and Ba were higher in monsoon tea.
- Concentrations of Pb, K, Cu, Zn, and Na were higher in pre- and post-monsoon tea.
- Sensory analysis revealed no metallic taste in environmental and commercial teas.

### A R T I C L E I N F O

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### A B S T R A C T

Plant-climate interactions affect the edible crop composition, impacting flavor, nutrition, and overall consumer liking. In this study, principal components analysis was used to assess the macro- and micronutrient metal concentrations in pre-monsoon (spring), monsoon (summer), and post-monsoon (autumn) tea (*Camelia sinensis* (L.) Kuntze) from Yunnan Province, China in 2014–2016. Statistical differences were observed ( $p = 1.35E-24$ ). Fe, Ca, Mg, Mn, Al, and Ba concentrations were higher in June (monsoon) than in March (pre-monsoon) and September (post-monsoon) compared to Pb, K, Cu, Zn, and Na, which were higher in March and September. Although Fe, Ca, Mg, Mn, Al, and Ba concentrations increased during the monsoon season, sensory analysis did not detect metallic taste in either minimally processed or farmer-processed (commercial) teas. This finding shows the seasonal differences in flavor were due to striking differences in organic chemical composition and concentration.

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**Abbreviations:** DI, deionized; PTFE, polytetrafluoroethylene; ICP/OES, inductively coupled plasma/optical emission spectroscopy; PCA, principal components analysis; ANOVA, analysis of variance; LOD, limit of detection; LOQ, limit of quantitation.

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### 1. Introduction

Changes in climate conditions have already affected crop yield, which has been extensively studied (Karl et al., 2009; Nelson et al., 2009; Kurukulasuriya and Rosenthal, 2013). Less understood and critical to human consumption is how these changes impact plant quality (Porter and Semenov, 2005; Teixeira et al., 2013; Ashenfelter and Storchmann, 2014), especially since extreme weather events are increasing in frequency and duration and

because consumers purchase plant-based products for their nutritional, health, and sensory properties (Kang et al., 2009; Lobell et al., 2011; IPCC, 2014; Melillo et al., 2014). Because tea (*Camelia sinensis* (L.) Kuntze) is the most consumed beverage in the world after water it makes a compelling system to study. Striking changes occur in secondary metabolite chemistry when the plant experiences heavy rainfall (Ahmed et al., 2014; Kowalsick et al., 2014). For example, we found that catechin concentrations decreased five days after the onset of the East Asian Monsoon rains (Ahmed et al., 2014). Although catechins are polyphenols, the concentration of total phenolics increased dramatically as did the antioxidant potential. Similarly, we measured remarkable differences in the seasonal and elevational concentrations of 550 volatile metabolites (Kowalsick et al., 2014; Kfoury et al., 2018a, 2018b). Some compounds such as camphene, 1-octen-3-one, 2-cyclohexen-1-ol and 2,6-dimethyl-3,7-octadiene-2,6-diol were detected in only pre-, monsoon, high or low elevation teas, while others such as sabinene, 5,6-epoxy- $\beta$ -ionone, (2E)-hexenol, and 2,3-dihydrobenzofuran increased or decreased by 100's of percent. Many of these metabolites are known flavor and/or nutritional compounds (Yuan et al., 2009; Holopainen and Gershenzon, 2010; Penuelas and Staudt, 2010). These findings are consistent with farmer perceptions and tea prices, since spring and high elevation teas are known to be more flavorful (Ahmed and Stepp, 2013; Ahmed et al., 2014; Han et al., 2017). The results above and those in this paper are based on minimally processed tea, namely, leaves that were microwaved in the field to stop enzymatic oxidation.

Fig. 1 shows the 2012 tea plants from Yunnan Province, China, which Ahmed and Kowalsick analyzed experienced the severest of droughts and wettest onset to the monsoon rains in decades (Boehm et al., 2016). For example, 5 mm of rain fell in February and March of 2012 compared to ~35 mm in 2014–2016. In contrast, twice the amount occurred in May 2012 versus 2014–2016.

In this paper, we report differences in the Ba, Cu, Fe, Li, Mn, Zn (micronutrient) and Ca, K, Mg, Na (macronutrient) concentrations as a function of rainfall and elevation for tea obtained from 2014 to 2016. Yunnan serves as our model test site because it experiences heavy precipitation every year due to the East Asian Monsoons, which provides an opportunity to learn how heavy rainfall affects metal concentrations within and across years. Although the effects of climate on metal concentrations is poorly understood (Myers et al., 2014), it is expected that changes in rainfall will change the solubility of the metals in soil making some more bioavailable and others less for plant uptake (Fung and Wong, 2001; Zhang et al., 2006; Meier and Leuschner, 2014). These essential nutrients, which can only be acquired through diet, are critical to human development (WHO, 1996; Otten et al., 2006; Soyak et al., 2007; Karak and Bhagat, 2010; Berdanier et al., 2014; Pohl et al., 2016; Karak et al., 2017).

## 2. Materials and methods

### 2.1. Tea leaves

Tea leaves from cultivar Yunnan #10 were collected from the same farm at two elevations (1790 m and 1180 m) in Menghai County, Yunnan Province, China. Pre-monsoon (March) and monsoon (June) samples were collected in 2014–2016. Post-monsoon (September) samples were collected in 2015 and 2016 only. The terminal bud plus two leaves were collected from five plants from four plots on three consecutive days ( $n = 60$ ). To prevent enzymatic oxidation, the leaves were microwaved in the field within 2 h of collection, hereafter called environmental tea, and mailed to Tufts University for analysis (Ahmed et al., 2014; Kowalsick et al., 2014). Upon receipt, samples were stored at  $-20^{\circ}\text{C}$  until analyzed.

### 2.2. Reagents

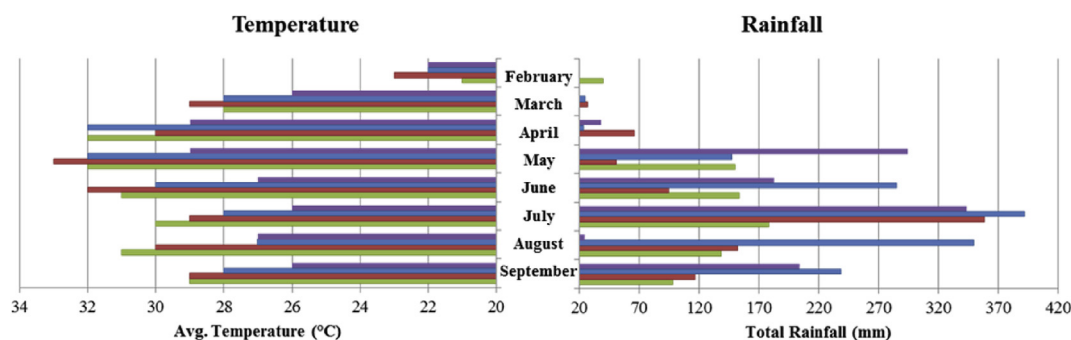
TraceMetal grade nitric acid ( $\text{HNO}_3$ ) and 30% ACS grade hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) were purchased from Fisher Scientific (Waltham, MA). Metal standards (1000 mg/L) in 2%  $\text{HNO}_3$  were purchased from Sigma Aldrich (St. Louis, MO). Deionized water (DI) was used to prepare standards and samples for analysis.

### 2.3. Sample extraction

Before sample digestion, the tea was dried at  $50^{\circ}\text{C}$  for 24 h then, ground to 140–200 mesh (105–74  $\mu\text{m}$ ) using a Kleco ball-mill (Visalia, CA). Two 1-g samples of each harvest day were weighed and put into separate covered glass beakers containing polytetrafluoroethylene (PTFE) boiling stones. 10 mL of  $\text{HNO}_3$  was added to the beaker followed by the dropwise addition of 1 mL  $\text{H}_2\text{O}_2$ . After 16 h, the sample was boiled on a hotplate until the digestate stopped smoking. After cooling, the digestate was diluted with DI water to bring the total volume to 25 mL, which was filtered using a 0.45  $\mu\text{m}$  PTFE syringe filter and stored at  $4^{\circ}\text{C}$  until analyzed. The same sample prep procedure was used to make reagent blanks. Note: six replicates (3 days  $\times$  2 extractions) were analyzed per harvest.

### 2.4. Inductively coupled plasma/optical emission spectroscopy

A Prodigy ICP/OES (Leeman Labs, Hudson, NH) was used to qualitatively and quantitatively analyze each sample. Instrument operating conditions were as follows: 1.2 kW power, 18 LPM of coolant gas, 0.2 LPM of argon auxiliary gas, 1.4 mL/min sample flow rate, 40 s sample uptake time and 32 psi nebulizer pressure. Samples were introduced using a concentric nebulizer. For qualitatively



**Fig. 1.** Temperature and rainfall in Menghai County, Yunnan Province, China, 2012, 2014–2016. Note: purple is 2012, blue, red, and green are 2014–2016, respectively (WorldWeatherOnline). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

analysis, each metal's wavelength was scanned for 10 s. For quantitative analysis, the resulting signal was an average of three snapshots in 1 s for axial and 5 s for radial detection.

### 2.5. Data analysis

An ICP/OES calibration curve was produced for the following metals: Al (308.215 nm, radial), Ba (455.403 nm), Ca (393.366 nm, radial), Cu (324.754 nm), Fe (238.204 nm), K (766.491 nm, radial), Li (610.364 nm), Mg (279.553 nm, radial), Mn (257.610 nm, radial), Na (589.592 nm), Pb (220.353 nm), Sr (407.771 nm) and Zn (202.548 nm) at the specified wavelengths in axial mode unless otherwise noted. Standards of each metal in 2% HNO<sub>3</sub> were serially diluted to concentrations found in the samples. Calibration curves were acceptable when  $r^2 \geq 0.99$ . The average metal concentration and percent relative standard deviation was based on six replicates of each sample variable. Principal components analysis (PCA) of the data was performed using Stata 15 (StataCorp, 2017). Analysis of variance (ANOVA) of the principal component scores, with Tukey post hoc tests, was conducted to assess statistical significance of the PCA separation.

### 2.6. Limit of detection and quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) for each metal was determined by a calibration curve and twelve reagent blanks. The LOD and LOQ are calculated as 3- and 10- times the standard deviation ( $\sigma$ ) of 12 reagent blanks divided by the slope ( $s$ ) of the calibration curve.

$$LOD = \frac{3\sigma}{s}$$

$$LOQ = \frac{10\sigma}{s}$$

### 2.7. Recovery

Accuracy was determined in two ways. First, samples were analyzed 3-times to measure the concentration of each metal in tea. Once known, we fortified each sample prior to digestion with reference standards, whose concentrations were approximately equal to that in the digestate, and analyzed them to determine recovery. Second, we fortified digested samples ( $n=6$ ) based on each metal's concentration in the digestate. For K, 1 mL aliquots were diluted 10:1 and then fortified with 1 mL of standard, whose concentrations were either 1- or 2-times that found in the diluted sample. These samples were further diluted to 25 mL prior to analysis. For the remaining metals, 1 (Mg, Ca, Mn), 2 (Al), 5 (Na, Fe), 10 (Zn, Ba, Cu), or 20 (Pb) mL of each digestate was added to 1 mL of reference standard, whose concentration was 1- or 2-times that of the digestate. These samples were diluted to 25 mL prior to analysis. The difference between the measured and fortified concentrations was used to determine accuracy.

### 2.8. Sensory analysis

The 2016, environmental and farmer processed, pre-, post-, and monsoon teas were analyzed by the Tufts University Sensory and Science Center's certified sensory panel. The Flavor Profile Method (Keane, 1992) was modified to evaluate each tea on a 7-point intensity scale, where one indicates no taste and seven strong taste. Infusions were prepared by brewing 3 g of each tea in 190 mL of

H<sub>2</sub>O at 90 °C for 3 min. The samples were coded, randomized, and served blind to each panelist one-at-a-time.

## 3. Results and discussion

Only a few studies have reported seasonal (Sud et al., 1995; Han et al., 2007; Ercisli et al., 2008) and elevational (Ramakrishna and Palmakumbura, 1987) changes of metals in tea. Toxic metals such as Al, As, Cd, Co, Cr, Ni and Pb have also been detected in tea (Ramakrishna and Palmakumbura, 1987; Ercisli et al., 2008; Zhao et al., 2017). In this study, ICP-OES was used to screen for potential metals in tea from the same farm at different elevations. Fig. 2 shows the precision and average concentration of metals detected above their LOQ, see method accuracy in Tables 1 and 2, for pre-monsoon (March), monsoon (June), and post-monsoon (September) samples at each elevation. Metal concentrations followed one of two trends. Namely, Fe, Ca, Mg, Mn, Al, and Ba concentrations were higher in June than March and September (Fig. 2a–f) compared to Pb, K, Cu, Zn, and Na (Fig. 2g–k), which were lower. Toxic metals, Al and Pb, were well below the Food and Agriculture Organization and World Health Organization limits for toxicity (JEFCA, 2011a, b). On average, measurement precision,  $4 \pm 2\%$ , and accuracy,  $101 \pm 3\%$ , were excellent.

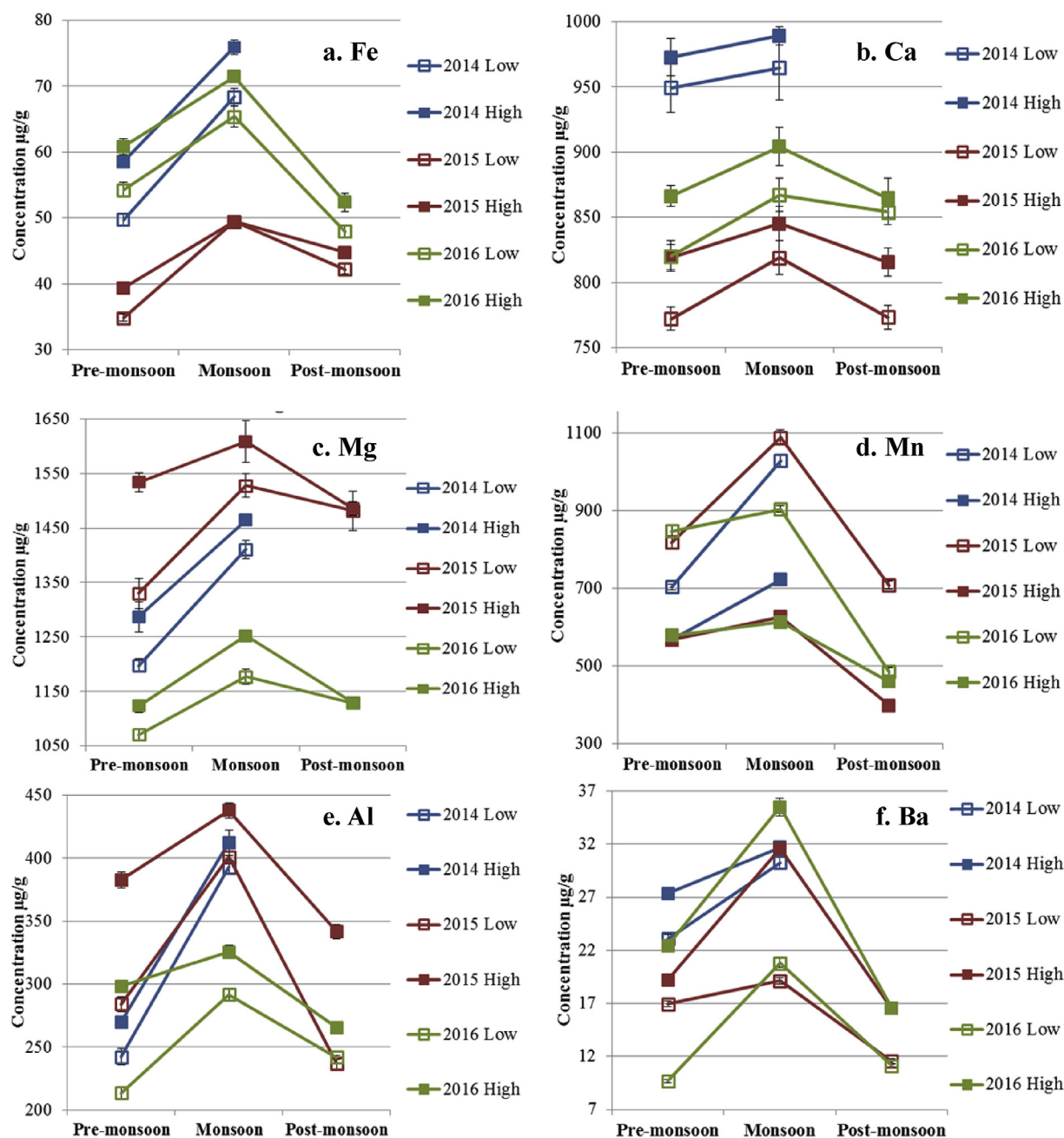
Also shown in Fig. 1 are the average monthly temperatures for the 2012 and 2014–2016 production period (WorldWeatherOnline). February was the coldest month; all other months were statistically the same ( $p > 0.05$ ). The temperature difference between the high and low elevation farms was  $\sim 4^\circ\text{C}$ , based on a  $6^\circ\text{C}$  decrease per 1000 m increase in elevation (NOAA, 2017). Pre-monsoon (February, March, April), monsoon (May, June, July), and post-monsoon (September) average monthly rainfall from 2014 to 2016 were statistically different from one another ( $p < 0.05$ ), see Table S1 in supporting information.

### 3.1. Multivariate analysis

Fig. 3 shows the PCA score plots illustrating the differences in micro- and macronutrient concentrations at both elevations for each harvest. The first two components capture 77% (Fig. 3a) and 70% (Fig. 3b) of the total variation in the data. The seasonal (square, circle, triangle) separation along PC1 was the same for both data sets (high elevation ANOVA  $F = 182.06$ ,  $p = 2.70 \times 10^{-22}$  and low elevation ANOVA  $F = 53.85$ ,  $p = 1.15 \times 10^{-12}$ ). The 2015 (red) samples were very different than the 2014 (blue) and 2016 (green) on PC2 at both elevations (high elevation ANOVA  $F = 76.408$ ,  $p = 1.44 \times 10^{-22}$  and low elevation ANOVA  $F = 16.59$ ,  $p = 4.0 \times 10^{-6}$ ).

Fig. 4 shows the principal components score plots with nutrient loadings when elevation is included. The first three principal components explain 83% of the variance. In Figs. 4a and 35% of the total variation is explained by seasonal observations on PC1 (ANOVA  $F = 105.12$ ,  $p = 1.35\text{E-}24$ ) while yearly data account for 31% of the total variation on PC2 (ANOVA  $F = 113.74$ ,  $p = 1.03\text{E-}25$ ). Findings from the PC1 Tukey post hoc test indicate pre- ( $p = 5.10\text{E-}9$ ) and post-monsoon ( $p = 5.10\text{E-}9$ ) are statistically different from the monsoon data and each other ( $p = 2.28\text{E-}8$ ). The PC2 Tukey post hoc test shows 2014 ( $p = 5.10\text{E-}9$ ) and 2016 ( $p = 5.10\text{E-}9$ ) are statistically different from 2015, but not from each other ( $p = 0.41$ ). In Fig. 4b elevation differences (filled vs. unfilled shapes) account for the remainder of the variation (PC3 ANOVA,  $F = 132.93$ ,  $p = 1.09\text{E-}19$ ).

The more positive the PC1  $r$  value is, the higher the metal concentration is in post-monsoon tea. In contrast, negative PC1  $r$  values correspond to monsoon teas and those near zero to pre-monsoon teas (Table 3). Only Pb was high in pre-monsoon tea, which is



**Fig. 2.** Fe (a), Ca (b), Mg (c), Mn (d), Al (e), Ba (f), Pb (g), K (h), Cu (i), Zn (j), and Na (k) concentration by season, elevation, and year. Since measurement precision was excellent, error bars for some data appear to be missing; its absence is an indication of data quality.

consistent with results reported by Han and coworkers (2007), who found Pb highest in pre-monsoon followed by post- and then monsoon teas. Since lead concentrations were low, the most likely source is atmospheric deposition.

Although other investigators (Fernandez-Caceres et al., 2001; Moreda-Pineiro et al., 2003; Milani et al., 2016) have measured Fe, Ca, and Ba concentrations in tea, no one has shown the effects of rainfall or elevation. The table shows these metals were higher in concentration in 2014/2016 as evidenced by the positive  $r$  values on PC2. Since each month's average temperature was statistically the same, differences in concentration are due to rainfall. Fe, a plant nutrient vital to plant health and growth, has been shown to

accumulate in the acidic soils (Natsen and Ranganathan, 1990; Broadley et al., 2012). Ca, also a plant nutrient, has been shown to increase in concentration in response to environmental stress (White and Broadley, 2003; Marschner, 2012). The exceptionally high  $r$  values suggest Fe (0.884) and Ca (0.765) are readily absorbed by the plant due to the higher rainfall in 2014/2016 compared to 2015.

In addition to rainfall, Mg, Cu, and Al are moderately dependent on elevation, which is depicted in Fig. 4b and Table 3. Positive and negative  $r$  values on PC3 correspond to high (filled shapes) and low (open shapes) elevations, respectively. Although plants generally grow faster at warmer temperatures (low elevation), all but Mg, Al,



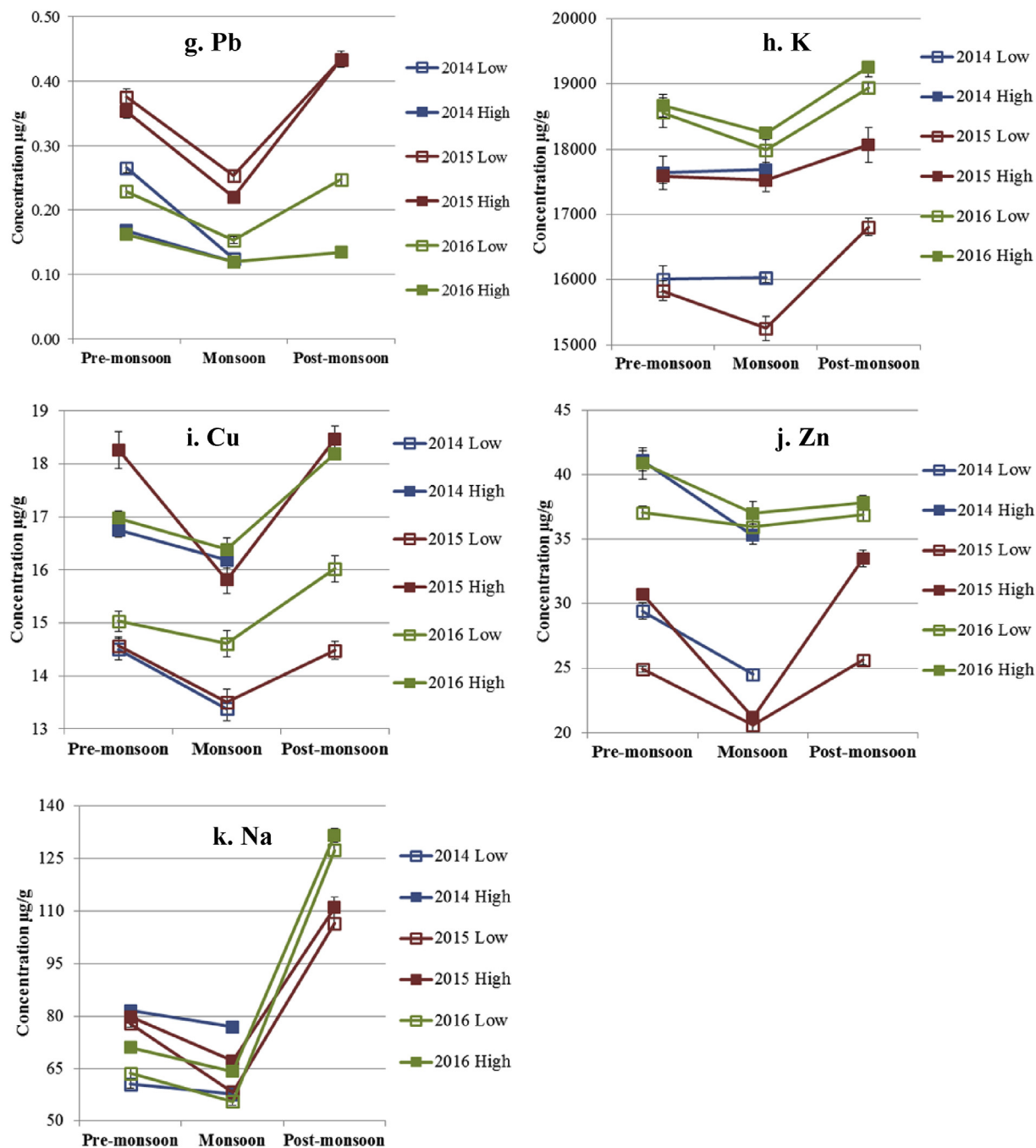


Fig. 2. (continued).

and Cu, which decreased in concentration, were unaffected by the 4 °C difference in temperature. For Mg, this finding is consistent with Shaul (2002), who found Mg concentrations decreased at warmer temperatures. Although Ramakrishna and coworkers (1987) measured Cu in Sri Lankan tea at different elevations, they did not report elevational comparisons.

### 3.2. Sensory evaluation

The 2016 environmental and farmer processed teas were evaluated by the sensory panel. Although pre-monsoon and monsoon teas differed significantly, the panelists observed no metallic taste

(intensity score = 1) in all teas. Based on our previous work (Ahmed et al., 2014; Kowalsick et al., 2014), differences in tea flavor can be attributed to changes in organic content with rainfall.

### 4. Conclusion

Tea from Yunnan Province, China, is an ideal model crop to investigate climate effects on agricultural crops. Yunnan Province experiences extremely low rainfalls in some years before the onset of the East Asian monsoon season, while tea plants collected from the same mountain at different elevations provide opportunity to study temperature effects from the same farms. Different from our

**Table 1**

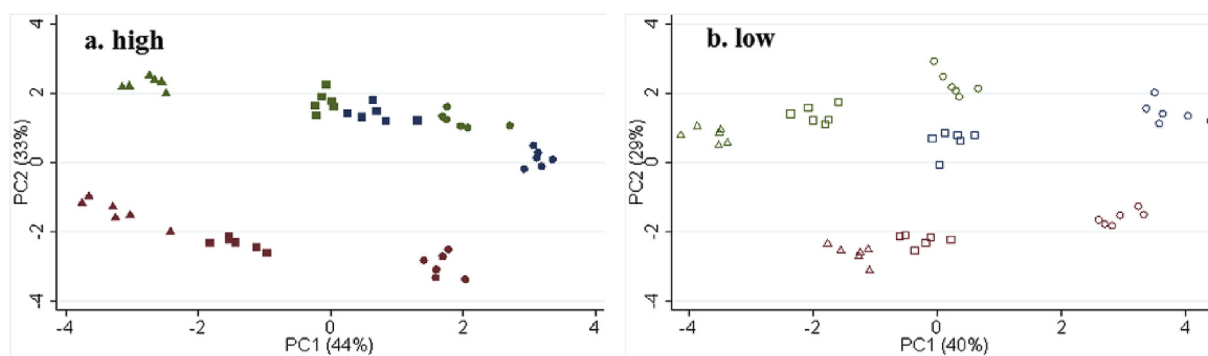
Percent recovery (and percent relative standard deviation, n = 3) of metals in samples fortified before digestion, see Fig. 2 for concentrations in actual samples.

Year	2014						2015						2016					
Altitude	1180 m			1790 m			1180 m			1790 m			1180 m			1790 m		
Season	pre	mon	post	pre	mon	post	pre	mon	post	pre	mon	post	pre	mon	post	pre	mon	post
K	96 (3)	100 (1)	101 (4)	100 (2)	102 (2)	103 (3)	101 (2)	99 (2)	104 (3)	102 (4)	99 (3)	100 (3)	99 (1)	104 (2)	104 (1)	101 (2)		
Mg	106 (3)	97 (3)	97 (5)	101 (1)	97 (1)	97 (3)	104 (6)	101 (3)	100 (6)	97 (2)	105 (2)	102 (3)	104 (2)	98 (3)	100 (2)	100 (2)		
Ca	106 (5)	106 (6)	105 (4)	97 (2)	102 (3)	101 (4)	99 (3)	98 (3)	96 (4)	102 (3)	98 (4)	97 (4)	99 (3)	100 (2)	101 (4)	102 (5)		
Mn	95 (2)	98 (4)	105 (3)	101 (3)	100 (3)	100 (5)	95 (4)	100 (5)	102 (6)	98 (3)	102 (4)	101 (2)	103 (5)	96 (5)	97 (4)	100 (4)		
Al	105 (6)	105 (4)	97 (4)	104 (6)	97 (1)	99 (3)	99 (4)	99 (4)	101 (3)	101 (4)	102 (5)	104 (4)	97 (1)	97 (3)	97 (4)	100 (2)		
Na	97 (5)	102 (3)	101 (3)	105 (2)	92 (5)	98 (4)	100 (4)	96 (3)	95 (5)	99 (6)	104 (6)	105 (4)	109 (3)	99 (6)	99 (4)	99 (4)		
Fe	104 (3)	99 (5)	107 (4)	98 (4)	96 (3)	104 (3)	104 (4)	97 (5)	97 (3)	102 (4)	97 (5)	98 (2)	98 (4)	100 (5)	100 (3)	103 (3)		
Zn	100 (5)	97 (4)	103 (5)	104 (5)	99 (4)	103 (2)	101 (4)	99 (3)	103 (2)	104 (5)	95 (3)	104 (2)	96 (3)	97 (6)	96 (6)	98 (4)		
Ba	104 (4)	106 (4)	102 (5)	105 (4)	101 (1)	98 (3)	99 (2)	100 (3)	102 (5)	98 (1)	98 (4)	105 (5)	104 (3)	103 (3)	100 (5)	104 (4)		
Cu	106 (4)	104 (4)	103 (2)	105 (2)	99 (3)	96 (5)	103 (3)	98 (5)	102 (4)	97 (3)	102 (3)	105 (4)	104 (4)	96 (2)	103 (3)	102 (2)		
Pb	104 (7)	101 (8)	102 (10)	98 (9)	102 (14)	103 (13)	103 (9)	95 (13)	94 (9)	108 (8)	105 (10)	98 (10)	103 (11)	104 (8)	101 (10)	105 (9)		

**Table 2**

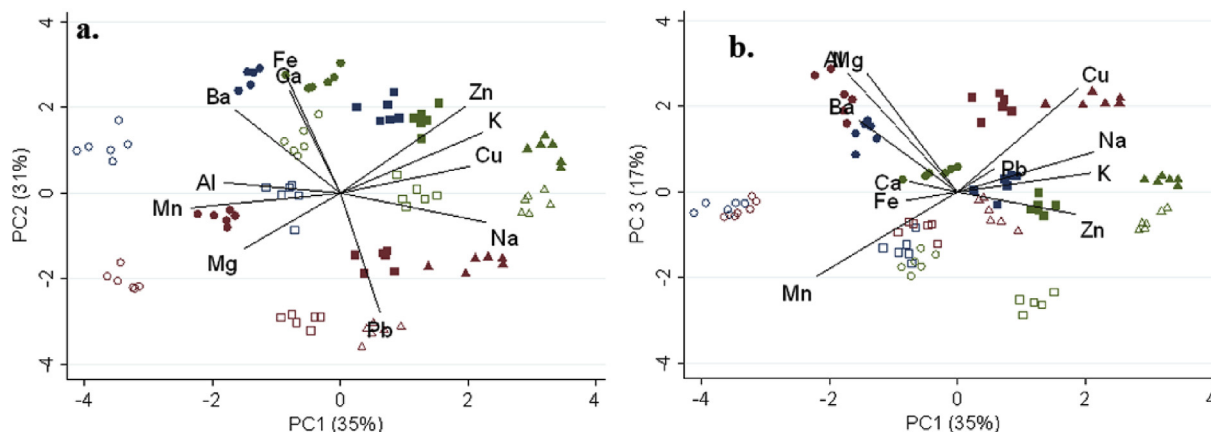
Percent recovery (and percent relative standard deviation, n = 6) of metals in samples fortified 1- and 2- times after digestion, see Fig. 2 for concentrations in actual samples.

Year	2014						2015						2016					
Altitude	1180 m			1790 m			1180 m			1790 m			1180 m			1790 m		
Season	pre	mon	post	pre	mon	post	pre	mon	post	pre	mon	post	pre	mon	post	pre	mon	post
K	100 (3)	100 (4)	103 (2)	100 (2)	100 (3)	102 (3)	103 (1)	101 (1)	100 (4)	98 (2)	102 (3)	99 (2)	100 (2)	101 (1)	99 (5)	97 (3)		
Mg	104 (1)	99 (2)	104 (3)	103 (1)	101 (4)	101 (3)	104 (2)	103 (2)	101 (1)	102 (3)	99 (4)	102 (2)	99 (4)	103 (3)	106 (2)	100 (2)		
Ca	101 (3)	105 (5)	98 (5)	101 (4)	99 (6)	104 (2)	98 (2)	97 (3)	99 (2)	100 (4)	97 (2)	102 (1)	97 (4)	101 (3)	104 (4)	102 (3)		
Mn	100 (3)	100 (1)	100 (3)	98 (3)	100 (2)	104 (3)	97 (2)	97 (2)	100 (2)	97 (3)	101 (5)	101 (2)	98 (3)	102 (2)	100 (1)	99 (5)		
Al	98 (5)	98 (4)	97 (3)	97 (3)	99 (3)	102 (4)	106 (3)	105 (2)	99 (5)	99 (3)	102 (4)	102 (2)	103 (3)	98 (4)	99 (1)	96 (3)		
Na	97 (4)	99 (2)	101 (4)	100 (3)	99 (4)	99 (3)	96 (3)	101 (4)	99 (2)	106 (1)	99 (3)	99 (2)	105 (5)	98 (2)	102 (3)	105 (2)		
Fe	100 (2)	102 (3)	99 (4)	101 (5)	97 (4)	99 (5)	104 (4)	105 (4)	97 (3)	101 (2)	98 (3)	96 (2)	102 (4)	96 (1)	103 (2)	102 (5)		
Zn	102 (4)	97 (3)	97 (5)	96 (3)	99 (2)	95 (5)	95 (3)	100 (2)	99 (2)	102 (1)	103 (5)	97 (4)	103 (4)	101 (3)	100 (4)	101 (2)		
Ba	99 (6)	102 (4)	97 (5)	104 (3)	97 (4)	101 (5)	102 (1)	97 (4)	97 (4)	104 (2)	101 (4)	104 (4)	101 (1)	99 (2)	97 (4)	97 (5)		
Cu	101 (3)	100 (4)	97 (5)	97 (5)	99 (3)	99 (4)	98 (3)	99 (2)	99 (1)	100 (3)	103 (3)	103 (1)	98 (3)	104 (5)	100 (3)	98 (2)		
Pb	96 (5)	104 (3)	99 (3)	105 (4)	105 (5)	104 (6)	101 (3)	101 (4)	105 (3)	101 (3)	99 (5)	100 (1)	99 (3)	101 (5)	109 (4)	99 (3)		
Ca	95 (3)	100 (2)	99 (4)	99 (3)	100 (4)	98 (4)	105 (5)	98 (4)	100 (2)	107 (4)	96 (2)	98 (2)	103 (3)	105 (3)	99 (1)	104 (2)		
Fe	97 (3)	98 (5)	96 (4)	98 (3)	104 (5)	104 (2)	108 (3)	107 (6)	104 (3)	104 (3)	102 (4)	105 (5)	102 (3)	94 (3)	98 (5)	101 (2)		
Zn	97 (4)	102 (4)	100 (4)	100 (4)	102 (3)	107 (4)	100 (5)	104 (4)	102 (5)	104 (6)	103 (2)	103 (2)	96 (4)	102 (2)	103 (3)	103 (4)		
Ba	99 (5)	95 (4)	97 (5)	104 (2)	101 (4)	101 (1)	107 (3)	103 (5)	101 (2)	107 (6)	104 (6)	99 (4)	98 (5)	99 (1)	96 (2)	102 (3)		
Cu	103 (4)	101 (5)	96 (5)	97 (5)	104 (3)	107 (5)	104 (2)	103 (3)	104 (3)	99 (4)	103 (3)	97 (2)	105 (4)	95 (3)	98 (4)	99 (2)		
Pb	100 (4)	98 (4)	102 (3)	105 (4)	102 (5)	106 (5)	100 (3)	109 (5)	102 (2)	98 (2)	98 (3)	105 (5)	102 (4)	102 (4)	104 (3)	98 (4)		
Ca	102 (4)	101 (4)	100 (4)	103 (3)	101 (1)	99 (5)	102 (4)	99 (3)	101 (1)	98 (2)	104 (4)	96 (3)	103 (2)	101 (1)	104 (3)	98 (3)		
Fe	98 (4)	102 (2)	101 (3)	105 (5)	96 (5)	101 (3)	110 (4)	103 (2)	96 (3)	104 (5)	97 (3)	98 (2)	106 (5)	101 (3)	104 (4)	105 (4)		
Zn	101 (4)	103 (2)	103 (4)	96 (3)	100 (4)	103 (3)	105 (4)	96 (3)	100 (2)	93 (4)	101 (1)	101 (4)	102 (1)	99 (2)	102 (4)	101 (2)		
Ba	95 (7)	105 (6)	101 (8)	98 (8)	97 (9)	103 (11)	97 (7)	102 (8)	97 (10)	103 (12)	108 (10)	106 (11)	109 (8)	103 (10)	103 (11)	102 (7)		
Cu	94 (7)	104 (7)	101 (7)	104 (10)	99 (6)	103 (8)	98 (11)	103 (12)	99 (11)	96 (9)	106 (8)	106 (7)	101 (9)	108 (12)	105 (9)	107 (8)		

**Fig. 3.** PCA plots by elevation. Notes: 1) pre-, monsoon, and post-are square, circle, and triangle on PC1 and 2) 2014, 2015, and 2016 are blue, red, and green on PC2, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

work on organics, where concentration changes were striking, metal concentrations, except Pb (−35%) and Ba (+40%), either increased or decreased by less than 25% between spring (pre-

monsoon) and summer (monsoon) seasons. In contrast, K and Na doubled in concentration between summer and fall (post-monsoon) seasons. Although changes in metal concentrations



**Fig. 4.** PC1 v. PC2 (a) and PC1 v. PC3 (b) plots with nutrient loading factors. Notes: 1) pre-, monsoon, and post-are square, circle, and triangle, 2) 2014, 2015, and 2016 are blue, red, and green, and 3) high and low are filled and unfilled shapes, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

**Table 3**

PCA correlations. Notes: When  $-0.75 \leq r \leq 0.75$  indicates strong correlation except PC1 where  $-0.25 \leq r \leq 0.25$  also specifies strong correlation for pre-monsoon data.

Variable	PC1		PC2		PC3	
	r	p-value	r	p-value	r	p-value
K	0.754	7.33e-19	0.451	3.97e-06	0.112	0.276
Na	0.771	3.92e-20	-0.217	0.033	0.235	0.021
Ca	-0.269	0.008	0.765	1.34e-19	0.060	0.559
Mg	-0.509	1.20e-07	-0.413	2.87e-05	0.695	4.17e-15
Mn	-0.795	4.31e-22	-0.112	0.281	-0.494	3.10e-07
Fe	-0.290	0.004	0.884	9.73e-33	0.050	0.626
Pb	0.209	0.040	-0.886	3.62e-33	0.137	0.182
Al	-0.621	1.54e-11	0.080	0.439	0.697	3.23e-15
Zn	0.666	1.28e-13	0.649	8.89e-13	-0.130	0.207
Ba	-0.554	4.79e-09	0.619	1.87e-11	0.420	0.0002
Cu	0.686	1.18e-14	0.199	0.052	0.611	3.78e-11

occur, extreme rainfall events do not affect plant quality as it relates to metals.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2018.12.075>.

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